Supplementary Information

Chronic monoacylglycerol lipase blockade causes functional antagonism of the endocannabinoid system

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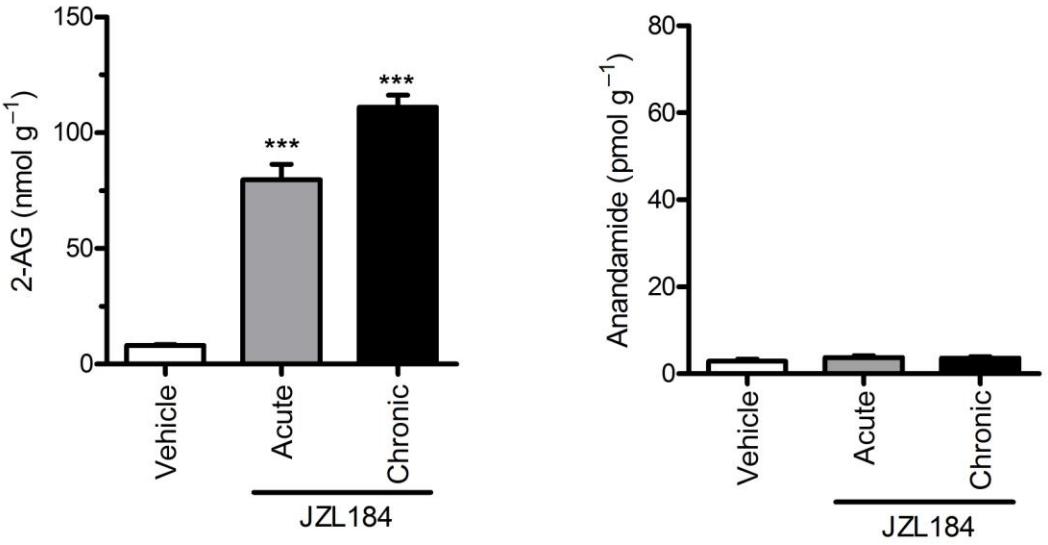
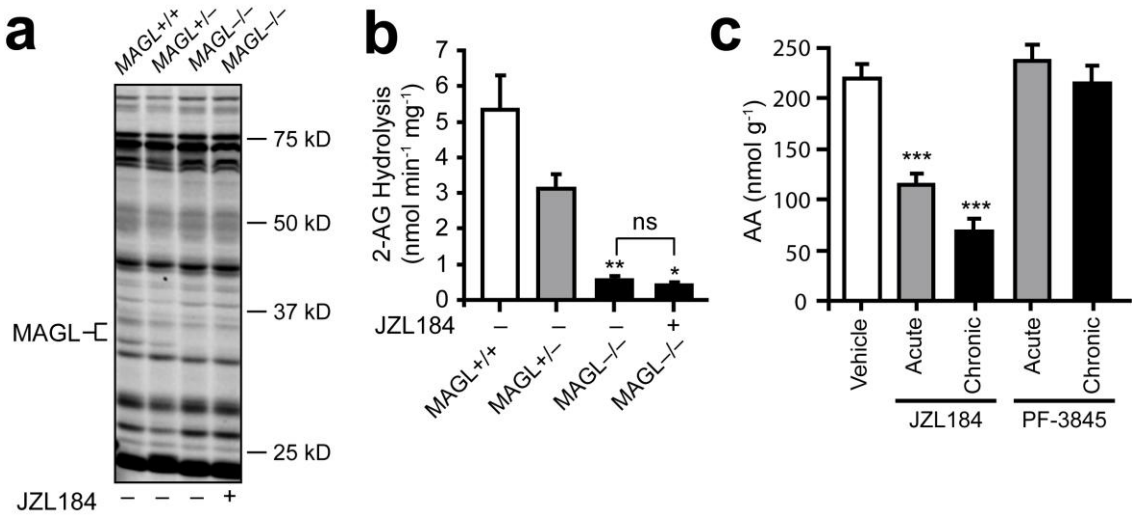
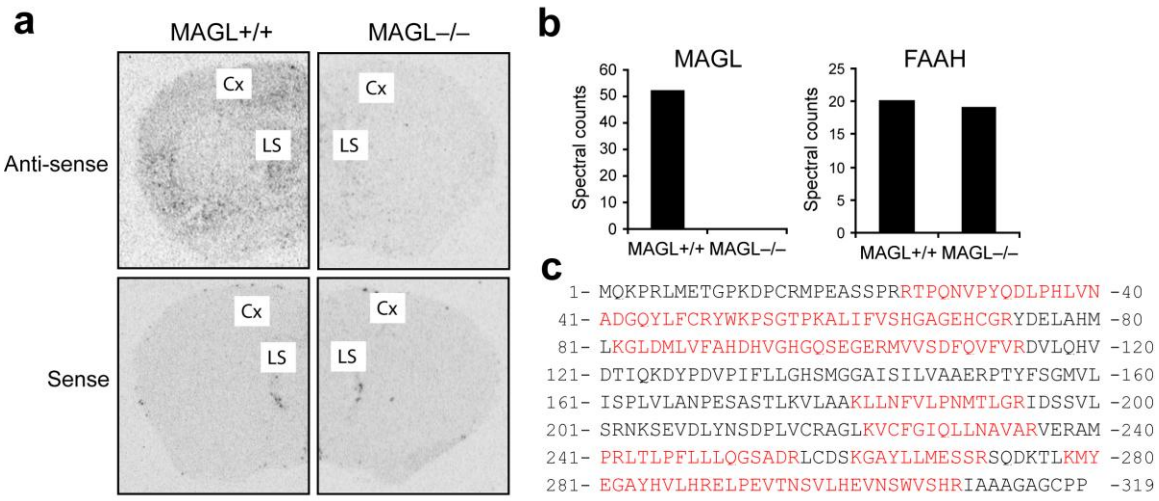


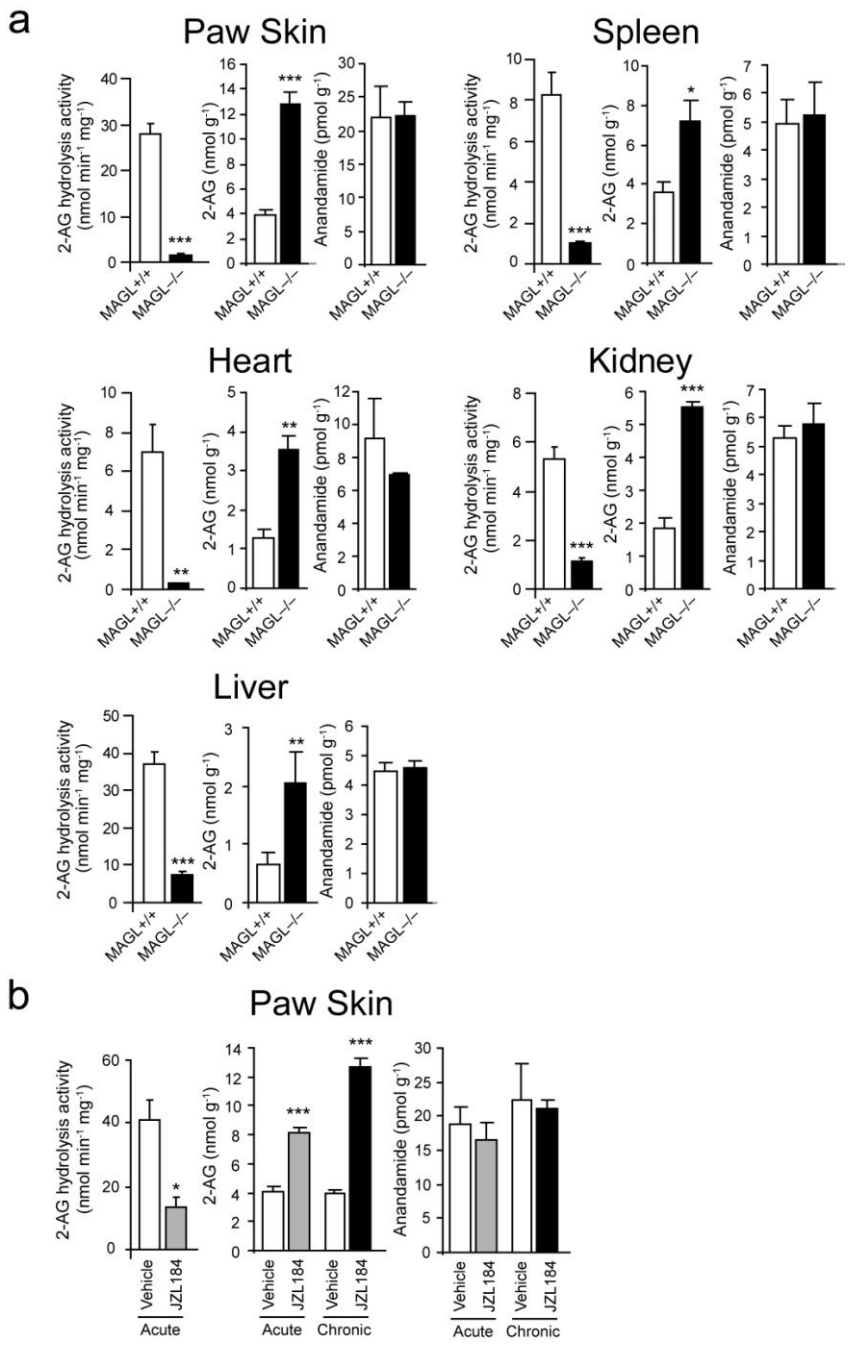
Supplementary Figure 1. Brain levels of endocannabinoids in mice treated acutely or chronically with JZL184 evaluated 26 hr after final dosing (acute dosing regimen: 40 mg/kg, i.p., 2 hr; chronic dosing regimen: six days, one dose per day). n = 5–6 mice per group. ***p < 0.001 versus vehicle-treated mice (Dunnett's test).



Supplementary Figure 2. Further characterization of endocannabinoid metabolism in mice with chronic disruptions of MAGL or FAAH**.** (**a**) Activity-based protein profiling of *MAGL*$^{+/+}$, *MAGL*$^{+/–}$, and *MAGL*$^{–/–}$ soluble brain proteomes with or without JZL184 (5 µM) pre-treatment. (**b**) 2-AG hydrolytic activities of *MAGL*$^{+/+}$, *MAGL*$^{+/–}$, and *MAGL*$^{–/–}$ soluble brain homogenates with or without JZL184 pre-treatment (1 µM); n = 4 mice per group. (**c**) Brain levels of arachidonic acid (AA) in mice treated acutely or chronically with JZL184 (acute dosing regimen: 40 mg/kg, i.p., 2 hr; chronic dosing regimen: six days, one dose per day, evaluated 2 hr after final dose) or PF-3845 (acute dosing regimen: 10 mg/kg, i.p., 2 hr; chronic dosing regimen: six days, one dose per day, evaluated 2 hr after final dose); n = 5–6 mice per group. Data are presented as means ± s.e.m. *p < 0.05, **p < 0.01, ***p < 0.001 versus vehicle-treated or wild-type littermate control mice (planned comparisons).

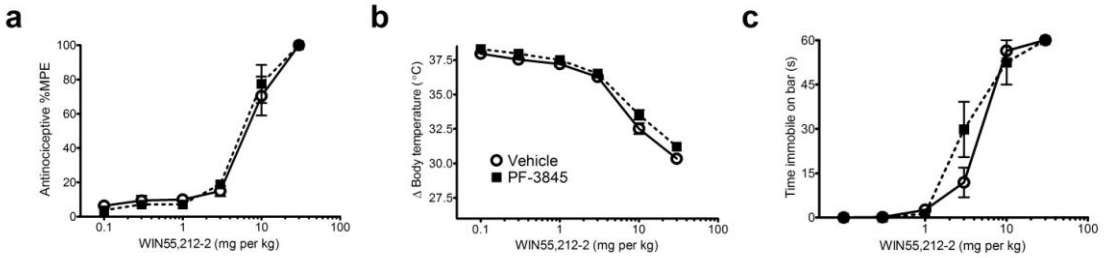


Supplementary Figure 3. Characterization of MAGL expression in *MAGL*$^{+/+}$ and MAGL$^{-/-}$ brains by i*n situ* hybridization and mass spectrometry-based proteomics. (**a**) *In situ* hybridization of *MAGL*$^{+/+}$ and *MAGL*$^{-/-}$ coronal brain sections with anti-sense and sense control [^{35}S]UTP-labeled MAGL riboprobes. MAGL mRNA was detected in MAGL$^{+/+}$ brains, but absent in *MAGL*$^{-/-}$ brains, which display a low signal equivalent to the sense probe control. Cx, cortex; LS, lateral septum. (**b**) Shotgun multidimensional LC-MS analysis of brain proteomes from *MAGL*$^{+/+}$ and *MAGL*$^{-/-}$ mice identified 52 spectral counts for MAGL peptides in *MAGL*$^{+/+}$ brains and 0 spectral counts for MAGL peptides in *MAGL*$^{-/-}$ brains. An equivalent number of FAAH spectral counts was identified in *MAGL*$^{+/+}$ and *MAGL*$^{-/-}$ brains. n = 3 mice per genotype. (**c**) MAGL peptides identified in *MAGL*$^{+/+}$ brain proteomes corresponded to 53% sequence coverage and included peptides ranging from amino acids 24–309 of the complete 319 amino acid sequence.

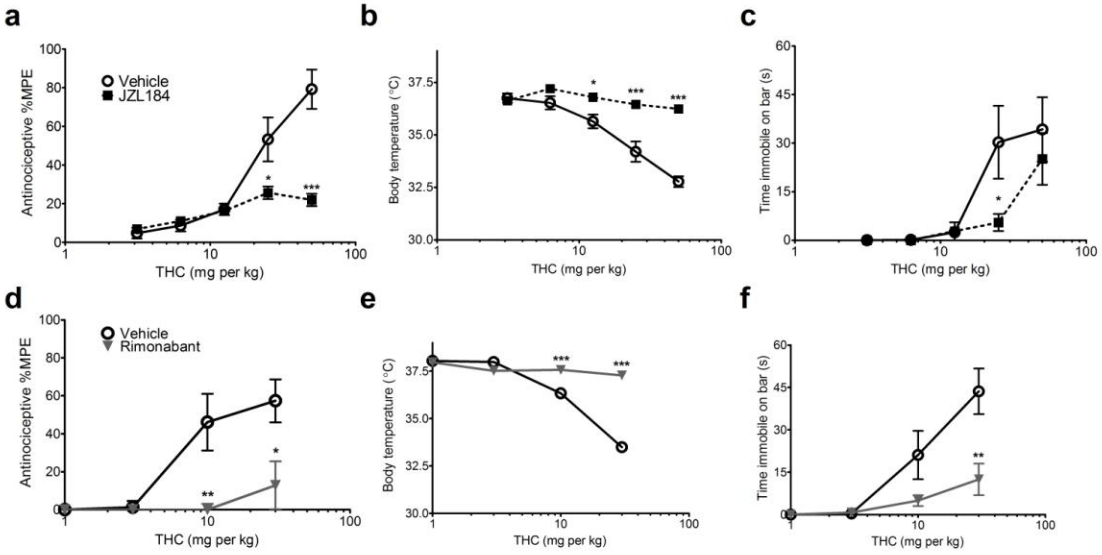


Supplementary Figure 4. 2-AG hydrolysis activity and metabolite levels in peripheral tissues of mice with chronic MAGL disruption. (**a**) 2-AG hydrolytic activities, 2-AG levels and anandamide levels in paw skin, spleen, heart, kidney and liver from *MAGL*$^{+/+}$ and *MAGL*$^{-/-}$ mice. n = 3–6 mice per genotype. (**b**) 2-AG hydrolysis activity of paw skin proteomes from mice treated acutely with JZL184 (40 mg/kg, i.p., 2 h) and paw skin

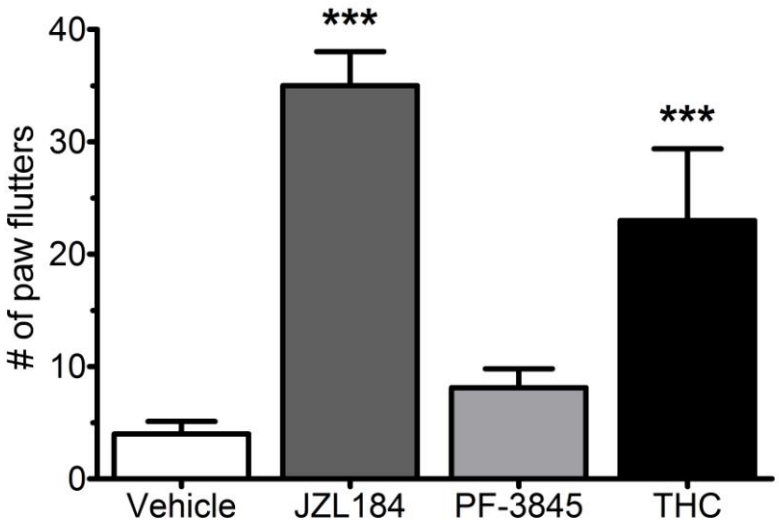
levels of 2-AG and anandamide from mice treated with JZL184 both acutely and chronically (six days, one 40 mg/kg dose per day, i.p., measurements made 2 h after final dosing). n = 3–4 mice per group. Data are presented as means ± s.e.m. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus wild-type littermate control mice (**a**) or vehicle-treated mice (**b**) (planned comparisons).



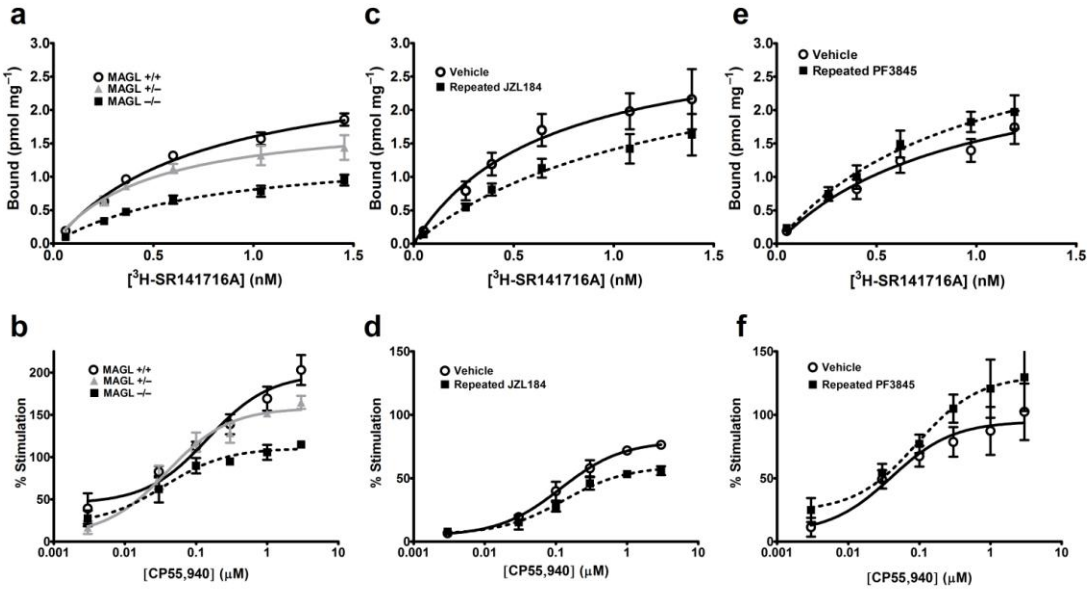
Supplementary Figure 5. Chronic treatment with the FAAH inhibitor PF-3845 (10 mg/kg, i.p., daily for 6 days) does not produce cross-tolerance to the CB_1 agonist WIN55,212-2. The antinociceptive (**a**), hypothermic (**b**), and cataleptic (**c**) effects of WIN55,212-2 were evaluated using a cumulative dosing paradigm, and no differences were observed at any dose for any behavior in PF-3845-treated mice compared to the vehicle treatment group. Data are presented as means ± s.e.m., n = 8 per group.



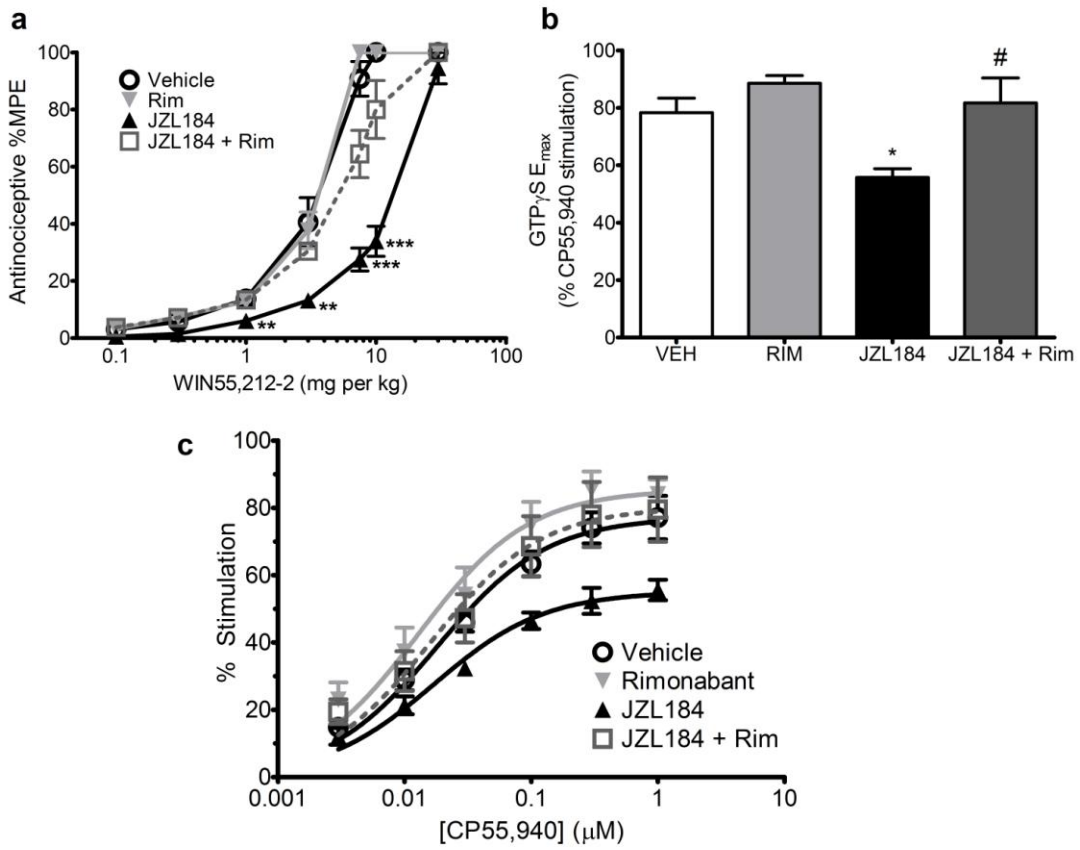
Supplementary Figure 6. Chronic treatment with JZL184 produces strong behavioral cross-tolerance to the antinociceptive (**a**) and hypothermic (**b**) effects of THC, but more limited cross-tolerance to the cataleptic effects of THC (**c**, where a significant difference was only observed at a single drug dose). Rimonabant (3 mg/kg, i.p.) blocked the antinociceptive (**a**), hypothermic (**b**), and cataleptic (**c**) effects of THC. Data are presented as means ± s.e.m. n = 7–8 per group. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus vehicle-treated mice (planned comparisons).



Supplementary Figure 7. Chronic JZL184 treatment produces physical dependence as judged by precipitated withdrawal responses to rimonabant. Rimonabant precipitated a comparable magnitude of withdrawal responses in mice treated chronically with JZL184 or a moderate THC dosing regimen (10 mg/kg, i.p., once per day for six days). Withdrawal responses were not observed in mice chronically treated with PF-3845. Data are presented as means ± s.e.m., with n = 6–8 per group in all studies. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ versus vehicle-treated or wild-type littermate control mice (Dunnett's post-hoc test). $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ versus respective acute drug treatment group (Bonferroni test).

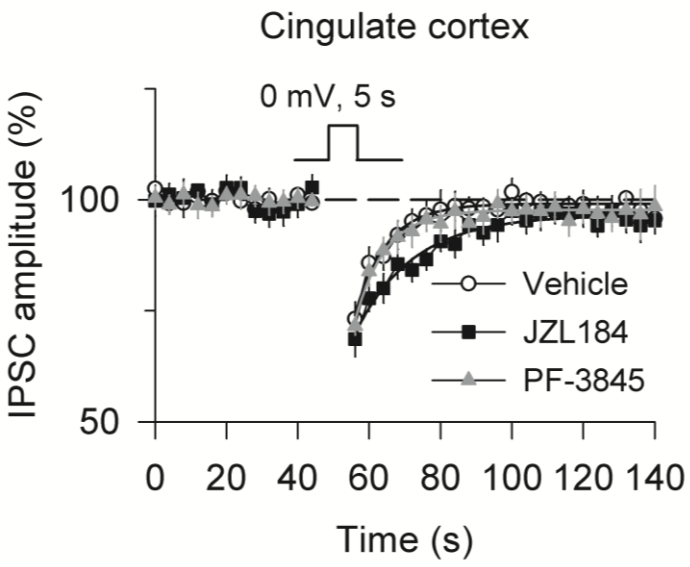


Supplementary Figure 8. Dose-response values and best-fit nonlinear regression curves for [^{3}H]-SR141716A (**a-**c) and [^{35}S]-GTPγS binding stimulated by the CB_1 agonist CP55,940 (**d-f**), from whole brain homogenates. (**a, d**) $MAGL^{-/-}$ mice show significantly reduced overall CB_1 receptor activation and membrane receptor numbers compared to wild-type controls. (**b, e**) Comparison of whole brain homogenates following chronic vehicle or JZL184 treatment demonstrates that JZL184 treatment decreases both the overall level of receptor signaling as well as the number of surface receptors available. (**c, f**) Chronic PF-3845 treatment does not produce reductions in overall CB_1 receptor binding or CB_1 stimulated G-protein activation. Data are presented as means ± s.e.m., n = 4 tissue samples per group, run in separate experiments with each point run in triplicate for GTPγS and duplicate for receptor binding.

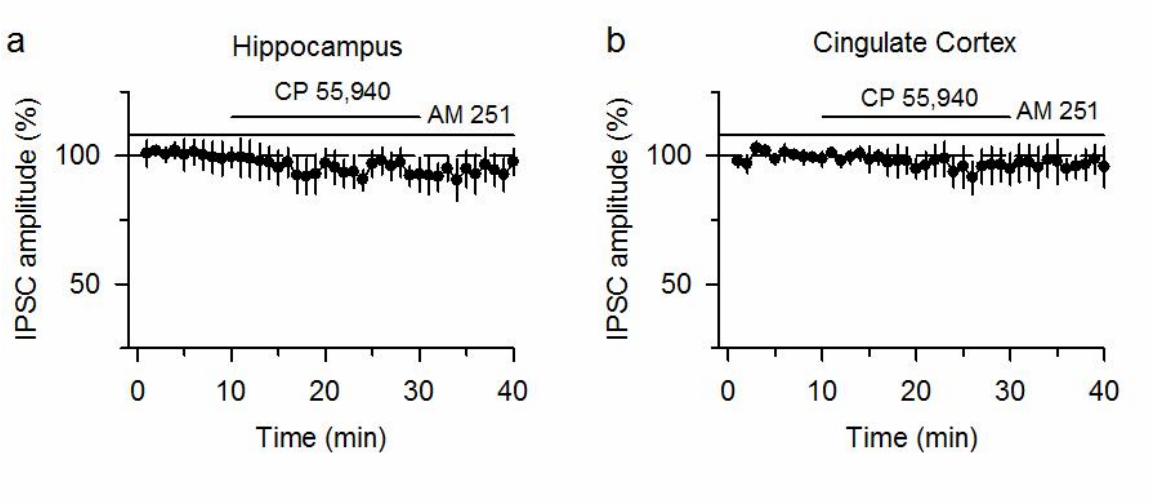


Supplementary Figure 9. Rimonabant (Rim) co-treatment blocks the cross-tolerance and receptor desensitization produced by chronic JZL184 treatment. (**a**) Chronic rimonabant (Rim; 3 mg/kg, i.p.) administered simultaneously with JZL184 (40 mg/kg, i.p.) significantly reduced antinociceptive cross-tolerance to WIN55,212-2, which was administered 48 h after final dosing with JZL184 and/or Rim. Rim alone did not significantly alter WIN55,212-2 activity after the 48 h washout period (also see **Supplementary Fig. 14**). n = 6 per group, $^{**}p < 0.01$, $^{***}p < 0.001$ versus vehicle and JZL184 + Rim groups at respective doses. (**b**) Summary of the nonlinear regression values for the estimated maximal CP55,940-stimulated [^{35}S]-GTPγS binding (E_{max}) of groups treated with chronic vehicle, rimonabant (10 mg/kg, i.p.), JZL184, or JZL184 + rimonabant. Values based on isotherms from (**c**). Rim co-administration prevented JZL184-induced reductions in whole-brain CB_1 receptor signaling function. Data are

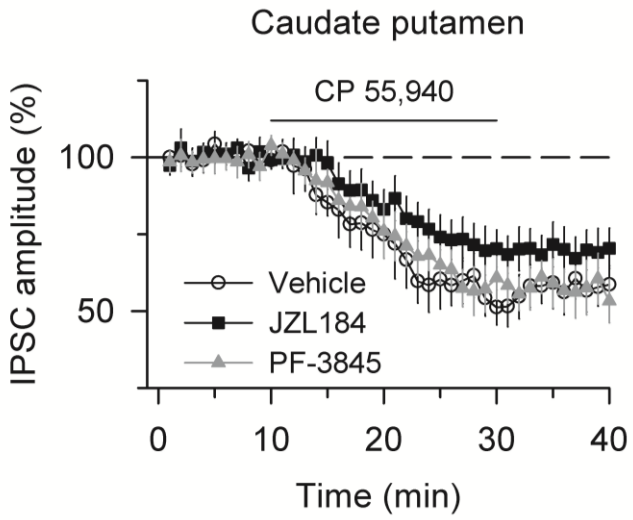
presented as means ± s.e.m., n = 4 samples per group, each sample run in triplicate. *p < 0.05 versus vehicle, $^{\#}p$ < 0.05 versus JZL184 group (Bonferroni corrected comparisons).



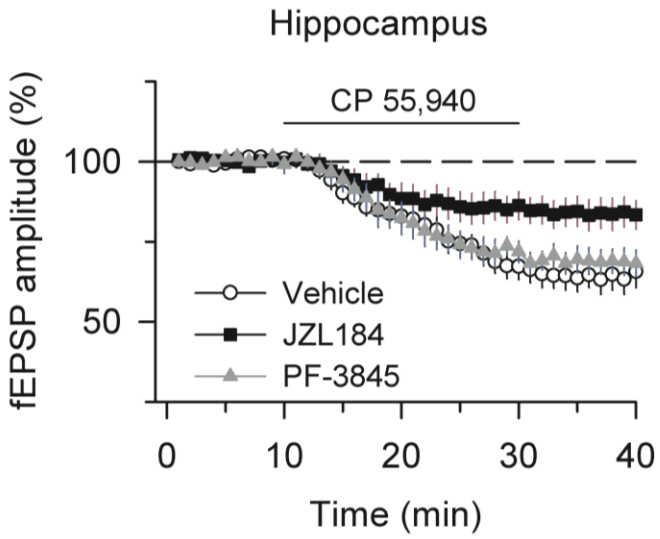
Supplementary Figure 10. Acute inhibition of MAGL, but not FAAH, potentiates DSI in layer V pyramidal neurons of the cingulate cortex. Bath perfusion of JZL184 (1 μM) prolonged DSI ($p < 0.05$ vs. vehicle), whereas bath perfusion of PF-3845 (10 μM) did not affect DSI ($p > 0.05$ vs. vehicle). ($n = 8–9$ per group). Neither JZL184 nor PF-3845 had significant effects on the magnitude of DSI. The lines superimposed are single exponential fitting curves of the decay of DSI.



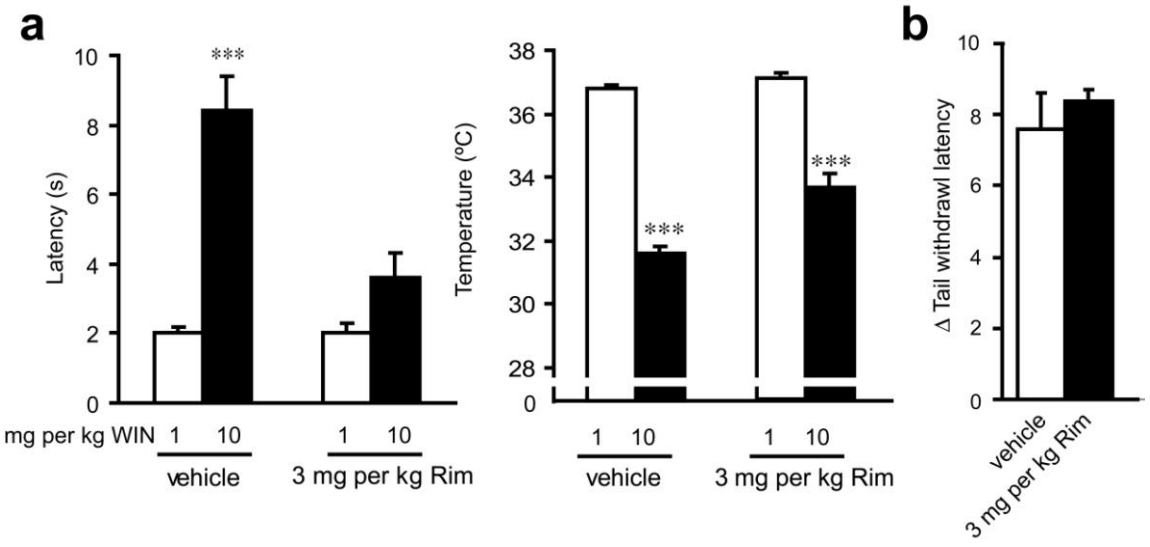
Supplementary Figure 11. CP55,940-induced depression of IPSCs in the hippocampus and cingulate cortex is mediated by CB_1 receptors. The CB_1 antagonist AM251 (2 µM) blocked CP55,940-induced depression of IPSCs in the hippocampus (a, n = 5) and cingulate cortex (b, n = 6) of vehicle-treated mice.



Supplementary Figure 12. Chronic treatment with JZL184 or PF-3845 does not significantly alter CP55,940-induced depression of IPSCs in medium spiny neurons of the caudate putamen (n = 7–8 per group, $p > 0.05$).



Supplementary Figure 13. Chronic JZL184 treatment reduces CB_1 agonist-mediated depression of glutamatergic excitatory transmission in the hippocampus. Bath application of the CB_1 agonist CP55,940 (3 µM) induced significantly less depression of fEPSPs in the CA1 region of the hippocampus from mice treated chronically with JZL184 compared to vehicle- or PF3845-treated mice (n = 8 per group, $p < 0.05$).



Supplementary Figure 14. Evaluating the duration of action for rimonabant (Rim) blockade of WIN55,212-2 (WIN)–induced behavioral effects. (**a**) Rim (3 mg/kg, i.p.) administered 26 hr prior to treatment with WIN (1 or 10 mg/kg, i.p.) effectively blocked the dose-dependent antinociceptive effect of WIN (left panel). In contrast, the hypothermic effect of WIN was incompletely antagonized 26 hr post-Rim dosing (right panel). (**b**) Rim (3 mg/kg, i.p.) administered 48 hr prior to treatment with WIN (10 mg/kg, i.p.) did not antagonize the antinociceptive effect of WIN. Data are presented as means ± s.e.m., with n = 4–6 per group in all studies. ***$p < 0.001$ for WIN 10 mg/kg group versus WIN 1 mg/kg group (planned comparisons).

Supplementary Table 1. Brain monoacylglycerol (MAG), N-acyl ethanolamine (NAE), and free fatty acid (FFA) levels from *MAGL*$^{+/+}$, *MAGL*$^{+/-}$, and *MAGL*$^{-/-}$mice; n = 4–6 mice per group. Data are presented as means ± s.e.m. *p < 0.05, **p < 0.01, ** p < 0.001 versus wild-type littermate controls.

	MAGL$^{+/+}$	*MAGL*$^{+/-}$	*MAGL*$^{-/-}$
MAGs (nmol/g)			
C16:0	5.1 ± 0.6	6.9 ± 0.8	11.1 ± 0.7 ***
C18:0	1.9 ± 0.7	1.9 ± 0.8	1.7 ± 0.2
C18:1	8.4 ± 1.5	12.7 ± 2.3	39.4 ± 11.0 *
C18:2	0.2 ± 0.1	0.3 ± 0.1	1.1 ± 0.2 **
C20:4 2-AG	10.8 ± 1.5	16.8 ± 1.2 *	147.8 ± 6.3 ***
C22:0	2.4 ± 0.3	2.1 ± 0.4	3.2 ± 0.3
C22:6	0.7 ± 0.1	1.1 ± 0.1 *	3.5 ± 0.4 ***
NAEs (pmol/g)			
C16:0	144.2 ± 7.4	198.0 ± 39.5	223.1 ± 63.3
C18:1	92.2 ± 4.6	118.9 ± 25.9	133.3 ± 31.6
C20:4 anandamide	2.6 ± 0.1	2.9 ± 0.4	2.6 ± 0.4
FFAs (nmol/g)			
C18:0	99.4 ± 10.4	118.2 ± 11.2	110.4 ± 9.7
C18:1	28.1 ± 2.7	34.2 ± 1.8	33.7 ± 4.0
C20:4 AA	109.7 ± 10.8	127.0 ± 5.0	38.5 ± 2.1 **
C22:6	8.7 ± 1.1	11.0 ± 0.8	8.2 ± 0.7

Supplementary Discussion

In order to test whether increased levels of 2-AG caused by chronic MAGL inhibition leads to a direct decrease in CB_1 function, we investigated whether concurrent administration of the CB_1 antagonist rimonabant and JZL184 could block cross-tolerance to WIN55,212-2. This study proved somewhat technically challenging because of the need to identify a dose of rimonabant that could sustain prolonged (ideally, 24 h) blockade of CB_1 receptors, but, at the same time, also permit washout within 48 h of final treatment to enable behavioral testing (of note, a previous report found that CB_1 receptor function begins to recover at three days following chronic treatment with cannabinoid agonists[1]). In a preliminary experiment, we confirmed that 3 mg/kg rimonabant (i.p.), which completely blocks the behavioral effects of acute JZL184[2], significantly attenuated the antinociceptive effects of WIN55,212-2 given 26 h, but not 48 h later (**Supplementary Fig. 14**). In contrast, the hypothermic effects of WIN55,212-2 were less effectively blocked by rimonabant given 26 h earlier. Consequently, we selected antinociception as our behavioral endpoint for the chronic rimonabant-JZL184 cross-tolerance study. In order to determine whether chronic rimonabant would also prevent CB_1 receptor desensitization caused by prolonged MAGL inhibition, the daily dose of rimonabant was increased to 10 mg/kg to ensure that CB_1 receptors were maximally antagonized throughout the course of the treatment period. The results of these behavioral and receptor adaptation studies, which are discussed in the main text of the manuscript and shown in **Supplementary Fig. 9**, support a model where chronic MAGL blockade produces functional antagonism of endocannabinoid signaling in the brain through sustained 2-AG elevations that tonically activate and eventually desensitize CB_1 receptors.

References

1. Sim-Selley, L.J.*, et al.* Prolonged recovery rate of CB1 receptor adaptation after cessation of long-term cannabinoid administration. *Mol Pharmacol* **70**, 986-996 (2006).

2. Long, J.Z.*, et al.* Selective blockade of 2-arachidonoylglycerol hydrolysis produces cannabinoid behavioral effects. *Nat Chem Biol* **5**, 37-44 (2009).